

Loss of inhibition over master pathways of bone mass regulation results in osteosclerotic bone metastases in prostate cancer

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Summary

Prostate cancer is the most common cancer among men in industrialised countries. Most patients with prostate cancer, however, will not die of it. As a result, many of them will experience symptomatic metastasis during the course of the disease. Prostate cancer has a high propensity to metastasize to bone. Unlike many other cancers prostate cancer cells induce a rather osteosclerotic than osteolytic reaction in the bone marrow by interfering with physiological bone remodelling. A proper understanding of the mechanisms of tumour cell-induced bone alterations and exaggerated bone deposition in prostate cancer may

open new and urgently needed therapeutic approaches in the field of palliative care for affected patients. In this review we focus on the central role of two major regulators of bone mass, the wingless type integration site family members (WNTs) and the bone morphogenetic proteins (BMPs), in the development of osteosclerotic bone metastases.

Key words: prostate cancer; bone metastases; osteosclerotic; metastases; WNT; bone morphogenetic proteins; Noggin

Introduction

Prostate cancer is the most common cancer affecting males in the industrialised world and is the second leading cause of cancer deaths among men. Median age at diagnosis is 68 years [1]. Non-detectable micro-metastatic disease may be present in up to 40% of patients [2] while 8–14% may have visible or symptomatic bone metastases at diagnosis [3]. Although radical prostatectomy has a significant impact on survival in localised disease, up to 30% of patients may need additional treatment in the form of androgen deprivation therapy (surgical or chemical castration) and up to 15% in the form of radiotherapy for local recurrence and previously undetected metastatic disease [4]. The gold standard for the treatment of locally advanced or metastatic disease consists of surgical or chemical castration. After a median period of 14–30 months the cancer cells may become resistant to castration [5]. During metastatic progression up to 90% of patients will experience bone metastases [6]. The median cancer specific survival time for castration resistant prostate cancer may be over 70 months [7]. Most of the patients with metastatic disease, therefore, will be at long-term risk for bone metastases with a more than 40% risk of skeletal complications (e.g., pain, spinal cord compression, fractures) [8].

A unique feature of prostate cancer is its consistent production of osteosclerotic rather than osteolytic bone metastases. Approximately 90% of the bone metastases in prostate cancer appear as an osteosclerotic clouding on plain x-ray films with a measurable increase in bone mineral density at the metastatic site [9]. This increase is a result of altered bone remodelling (increased bone resorption and deposition), whose balance has been tipped towards increased bone deposition upon invasion of the bone marrow by cancer cells.

Interaction with cancer induced bone remodelling is an interesting therapeutic approach in the palliative care of patients with bone metastases. For example, bisphosphonates, which block osteoclast activity and thereby bone resorption, have been shown to be highly effective in the prevention of skeletal complications in prostate cancer [10]. They have not however shown to decrease overall or cancer specific mortality so far. Further research is therefore needed on the prevention and treatment of bone metastasis in prostate cancer. One precondition for the development of new therapeutic strategies increasing bone health in affected patients is an understanding of normal and pathological bone turnover in general and of the mechanisms leading to os-

teosclerotic bone metastasis in particular. Molecular pathways responsible for increased bone deposition have been unravelled in hereditary diseases and animal models, as well as in cancer models, and may lead the way to new therapies.

Here we review the molecular control of two pathways responsible for increased bone deposi-

tion at the metastatic site, focusing on the role of wingless type integration site family members (WNTs) and bone morphogenetic proteins (BMPs) in prostate cancer.

Physiological and pathological bone remodelling

Physiological bone resorption and formation occur in a balanced sequence, the replacement of old bone with new bone maintaining the skeleton's structural integrity throughout adult life [11]. Osteoblasts, which arise from mesenchymal progenitor cells in the bone marrow and periosteum, are responsible for bone formation, whereas osteoclasts, which are derived from the mononucleated hematopoietic cell lineage, are responsible for bone resorption [12, 13].

Mechanical stress, cytokines, central nervous system stimuli and hormones determine the bone turnover rate [12, 14]. Osteoblasts play a predominant role in inducing the recruitment and activity of osteoclasts at the site of bone resorption. Colony stimulating factor-1 (CSF-1) [15] and receptor activator of NF-kappaB ligand (RANKL) [16, 17] are the two main factors produced by osteoblasts, each of which is necessary to generate osteoclasts. Osteoprotegerin (OPG), acts as a decoy receptor for RANKL and prevents RANKL from binding to the RANKL receptor (RANK) on the osteoclast progenitors, thus inhibiting osteoclast recruitment and, consequently, bone resorption [18, 19]. Most osteotropic factors, such as parathyroid hormone (PTH), PTH-related peptide (PTHrP), 1,25(OH)₂-vitamin-D3 and oestrogens, and local cytokines (interleukins), act indirectly on osteoclast generation by modulating RANKL expression in cells of the osteoblast lineage [20, 21]. Systemic factors, such as PTH, oestrogens, prostaglandins and cytokines, modulate osteoblast recruitment [22] to fill the gap created by osteoclasts. Most local mitogenic factors such as bone morphogenetic proteins

(BMPs), insulin-like growth factor (IGF) and transforming growth factor β (TGF- β), are embedded within the calcified matrix [23] and released from the bone matrix and activated during bone resorption. Stromal cells and their paracrine cytokine profiles play an important and probably underestimated role in modulating osteoblast differentiation and activity [24, 25].

The profile of hormones and cytokines affecting osteoblast recruitment and function is, therefore, strongly dependent on bone turnover and influenced by the surrounding stroma, stromal cells and bone marrow cells. Infiltrating cancer cells interfere with the tight control of bone remodelling by changing the cellular environment and disturbing the autochthon cytokine profile. In prostate cancer this results in increased bone deposition. Histological analysis of these metastatic bone deposits and serological analysis of bone turnover demonstrates different degrees of an increased and mixed osteolytic and osteosclerotic activity during the course of the disease [26, 27]. In physiological bone remodelling the activation of osteoblast-driven bone deposition is dependent at least in part on the preceding osteolytic activity, a phenomenon known as coupling [28]. Hence the increased osteolytic activity in prostate cancer metastasis may represent a precondition for the generation of osteosclerotic metastases. Finally, osteosclerotic metastases are prone to pathological fractures due to deposition of bone of the woven (immature or embryonal) type, which is much less mechanically competent than the lamellar (mature) type [29].

Regulation of bone mass: Lessons learned from sclerosing bone disorders

Regulation of bone mass by WNT-signalling

Research on hereditary disorders associated with increased bone mass has provided considerable insight into the regulation of osteoblast activity and increased net bone deposition [30]. Mutations in sclerostin (SOST) are associated with the rare familial disorders sclerosteosis and van Buchem disease [31, 32]. SOST is an inhibitor of the low-density lipoprotein receptor-related protein 5 (LRP5), an activating co-receptor of the transmembrane receptor frizzled, which itself is

activated by WNTs [33]. The WNT family of secreted proteins participates in multiple developmental events during embryogenesis and mediates bone development in the embryo and promotes bone production in the adult [reviewed in 34, 35]. Canonical WNT-signalling induces nuclear transcription of genes, inducing bone formation by stabilisation of the otherwise degraded intracellular β -catenin, which activates the transcription factor T-cell factor/lymphoid enhancer factor-1 (TCF/LEF-1) [36]. Osteotropic factors induced

by WNT signalling include OPG and endothelin-1 (ET-1) [37, 38]. As outlined above, OPG acts as a decoy receptor of RANKL, thereby preventing the binding of RANKL to RANK and inhibiting the activation of osteoclast function. ET-1 stimulates osteoblast proliferation and differentiation and decreases the motility and activity of osteoclasts [39, 40]. Loss of LRP5 inhibition by SOST mutations, therefore, results in increased bone formation. The same is true for members of the dickkopf (DKK) family that inhibit LRP5 in a manner similar to that of SOST and for activating mutations in the LRP5 gene, which are responsible for the clinical entity of high bone mass syndrome [41]. More WNT inhibitors and components of the WNT signalling pathway may influence bone mass, but have not yet been linked to clinical disorders [36].

Regulation of bone mass by BMP-signalling

Camurati-Engelmann disease is a rare clinical disorder associated with cortical thickening of the long bones due to activating mutations in the TGF β 1 gene [42]. TGF β 1 is the paradigmatic member of the TGF β -superfamily, a family of secreted proteins that includes the three isoforms of TGF β (TGF β 1, TGF β 2 and TGF β 3), Activin, Nodal, Mullerian-inhibiting substance, and the growth differentiation factor and BMP families. Fibrodysplasia ossificans progressiva, the most disabling condition of progressive heterotopic ossification in humans, is caused by a recurrent heterozygous missense mutation in activin receptor IA, a BMP receptor [43]. Inactivating mutations in LEM domain-containing protein 3 (LEMD3), a protein binding the inner nuclear membrane, antagonizes TGF β and BMP signalling, resulting in osteopoikilosis characterised by spotted bones due to osteosclerosis [44]. The largest family of cytokines in the TGF β -superfamily is the BMP family, which has been named for their ability to

induce ectopic bone formation and has been shown to be essential for bone development in general and more specifically for osteoblast proliferation and differentiation [45]. Knockouts of BMP2, 3, 4, 5, 6 and BMP-7 all result in pathological skeletal development [46]. TGF/BMP signalling is initiated by ligand-dependent homo- or hetero-dimerisation of membrane-bound receptor types I and II [47]. The ligand-dependent receptor-dimerisation of type I and II receptors activates the intracellular receptor type I kinase, followed by phosphorylation of intracellular SMAD-signalling molecules. Phosphorylated SMADs oligomerise in different combinations and translocate to the nucleus [48], inducing gene expression important for bone formation [49]. Extracellular BMP-antagonists (BMPA) sequester BMPs in the extracellular space by direct association with BMPs. Antagonism of BMP activity by the BMPAs Noggin and Chordin is critical for embryonic chondro-osteogenesis and joint formation [50, 51]. Osteoblast-targeted over-expression of Noggin [52] and the BMPA Gremlin [53] results in osteopenia due to impaired osteoblast recruitment and function, indicating that extracellular control of BMPs is also essential in adult life in maintaining the balance between bone resorption and formation in bone remodelling. Synthesis and secretion of BMPAs is BMP-dependent, showing that a perfect balance between BMPs and BMPAs is necessary to achieve optimal bone mass [54].

In conclusion, WNT and TGF/BMP signalling seem to play an important role in the regulation of bone mass, as evidenced by genetic diseases and animal models. Interestingly, both pathways are able to induce the runt-related transcription factor 2 (RUNX2), which is essential for bone formation [55], indicating an important and converging role in regulating bone mass.

Mechanisms of osteosclerotic bone metastasis

Contribution of WNT-signalling to osteosclerotic bone metastasis

The importance of WNTs and WNT inhibitors secreted by tumour cells was first identified in multiple myeloma [56], a disease that causes severe osteolysis with both enhanced bone resorption and suppressed bone formation. One of the factors responsible for this enhanced osteolytic activity was later identified as the WNT-inhibitor DKK-1 [56]. DKK-1 is also upregulated in localised prostate cancer but decreases later in metastatic disease [57]. Prostate cancer cells that induce osteolytic experimental bone metastases maintain expression of high levels of DKK-1, whereas prostate cancer cells inducing osteosclerotic metastases do not [58]. Osteolytic disease and metastases expressing high levels of DKK-1

have a worse clinical course than those that do not [57]. It is possible that DKK-1 is involved in the initial and increased osteolytic activity that enables the osteosclerotic activity (coupling) of osteoblasts, whereas at a later stage the loss of DKK-1 expression promotes formation of osteosclerotic bone metastases by unopposed activation of the WNT-signalling pathway [59]. SOST, the other inhibitor of WNT signalling mentioned above, has a not yet defined role in bone metastases. Measurements of SOST expression in prostate cancer cell lines did not, however, show a difference between osteolytic and osteosclerotic cancer cells [58]. In addition to the unopposed WNT signalling by loss of DKK-1, increased levels of WNT and β -catenin have been measured in clinical samples of prostate cancer and have been

associated with aggressive cancer behaviour [60, 61]. Moreover, blocking of WNT-signalling in a cell line that induced experimental osteosclerotic bone metastases converted that cell line into a highly osteolytic one [59], demonstrating that WNT-signalling plays an important role in the formation of osteosclerotic bone metastases. As outlined above, the canonical WNT-signalling pathway induces the transcription of OPG and ET-1, both factors associated with increased bone mass. Elevated levels of OPG in the serum of prostate cancer patients are indicative of prostate cancer bone metastases [62]. OPG is expressed at high levels in prostate cancer osteoblastic metastasis [63] and overexpression of OPG in prostate cancer cells results in increased bone volume when these cells are grown in the bone [64].

ET-1 appears to play a similar role in the generation of osteosclerotic bone metastases, as evidenced by its experimental inhibition of DKK-1 secretion [65]. Its causal role in evoking an osteosclerotic reaction in bone metastasis of mammary cancer is well recognised [66], and a similar role has been postulated in prostate cancer [39, 67].

Contribution of BMP-signalling to osteosclerotic bone metastasis

A systematic work-up of the expression of members of the TGF β -superfamily in prostate cancer and in prostate cancer cell lines that induce experimental osteolytic and osteosclerotic bone metastases was recently published by our laboratory [58, 68]. Prostate cancer cell lines, either osteolytic or osteosclerotic, expressed at least one sort of BMPs. Consistent with other reports [69], BMP6 was predominantly expressed by osteosclerotic prostate cancer cell lines, further supporting a role for BMP6 in the development

of osteosclerotic bone metastases in prostate cancer. The most striking finding was the restricted expression of the BMPA Noggin to cell lines inducing osteolytic bone metastases. Re-expression of Noggin in prostate cancer cells inducing osteosclerotic experimental bone metastases significantly reduced the osteosclerotic capacity of these cells and normalised the bone structural parameters in the bone metastases to normal control values conformable with a return to physiological bone remodelling. As a consequence, in this experimental system the stimulation by BMPs derived either from cancer cells or from the bone microenvironment resulted in an exaggerated osteoblast response only when not antagonised by the BMPA Noggin. Moreover, Noggin re-expression reduced the number of osteoclasts at the bone metastatic site. This study was the first to demonstrate that the unopposed BMP signal contributes to the osteosclerotic phenotype of prostate cancer metastases *in vivo*.

To sum up, loss of the inhibitors DKK-1 and Noggin promotes an unsuppressed WNT and BMP signal at the bone metastatic site and probably contributes fundamentally to the formation of osteosclerotic bone metastasis in prostate cancer. Expression of Noggin in experimental osteosclerotic bone metastasis represses the osteosclerotic response irrespective of which signalling pathway is neutralised. Thus, it is possible that none of these signalling pathways is sufficient *per se* to evoke an osteosclerotic response, but rather they must act in concert. This hypothesis is further substantiated by a recent experimental study demonstrating that the WNT and BMP signalling pathways act together in provoking the osteosclerotic reaction of prostate cancer bone metastases [70].

Therapeutic implications of WNT and BMP signalling

Bones affected by either osteolytic or osteosclerotic metastasis are more prone to pain and pathological fractures. The demonstrated role of WNT signalling in bone and its involvement in the response of bone to prostate cancer cells prompts investigation of novel drugs targeting the WNT pathway for successful prevention of skeletal complications. The inhibitory effect of Noggin on the osteoblast and osteoclast response in bone metastatic lesions may reduce the incidence of pathological bone fractures. Hence, inhibiting BMP signalling by a substance similar to Noggin could prove to be useful as an adjuvant drug in the treatment of metastasis-induced skeletal complications.

Although basic research has brought us closer to an understanding of the mechanisms of cancer-induced bone alterations, the therapeutic implica-

tions are still evolving. Potential agents, such as the ETAR antagonist atrasentan, are not yet showing conclusive results in clinical phase III trials [71, 72]. More substances are being tested, but only a few attack the molecular pathways described above [73]. Continued research is clearly warranted and ultimately needed for patients suffering from prostate cancer bone metastasis.

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